4.2

Anabolic and catabolic responses of human articular chondrocyte from aged donors to culture under low oxygen tension

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Purpose: Aging is known to negatively modulate the metabolism of chondrocytes. This study was undertaken to investigate whether culturing human articular chondrocytes from aged donors (HAC) at low oxygen tension during expansion in monolayer or re-differentiation in pellets would improve their chondrogenic capacity and reduce the expression of specific catabolic mediators.
Methods and Materials: HAC isolated from the cartilage biopsies of four patients (mean age 65 years) were expanded either at 20% or 5% oxygen tension in 10%FBS medium containing TGFb-1/FGF-2/PDGF-BB. Post-expanded cells where then cultured as pellets in medium promoting chondrogenesis under the two different oxygen tensions. The generated cartilaginous tissues were assessed histologically (Safranin-O), biochemically (glycosaminoglycans -GAG- and DNA), immunohistochemically (collagen-II) and by RT-PCR (collagen-II, aggrecan, MMPs and TIMPs).
Results: HAC expanded under the two oxygen tensions produced tissues with similar quality and GAG/DNA contents following differentiation under 20%O~2~. Instead, differentiation at 5%O~2~ (vs 20%O~2~) of HAC expanded at 20% O~2~ improved the intensity of staining for GAG and collagen-II, the GAG/DNA content (2.8-fold) and the expression of aggrecan (8.5-fold) and collagen type II (86.6-fold) mRNA. Moreover, pellets cultured under lower oxygen tension expressed lower MMP-1 (7.7-fold) and MMP-13 (3.5-fold) and higher TIMP-1 (3.6-fold) mRNA.
Conclusions: Low oxygen tension applied during re-differentiation in 3D-culture not only enhances matrix production but also reduces the expression of catabolic mediators by HAC, whereas it does not appear to have marked effect if applied during expansion. 3D culture at a more physiological oxygen level might be useful to enhance the outcome of cartilage engineering techniques in aged individuals.

4.3

Change of energy metabolism after monolayer culture of chondrocytes

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Purpose: For autologous chondrocyte repair, chondrocytes are usually expanded in monolayer where it is known they dedifferentiate. Here we report on the changes in energy metabolism of chondrocytes after culture in monolayer and then after return to 3-D culture systems.
Methods and Materials: Chondrocytes were isolated by enzymatic digestion from metacarpal-phalangeal cartilage of 18-24 month bovine steers. They were then either cultured in alginate beads for the whole experiment or else in monolayer on microcarrier beads for 10 days. The monolayer cultures were then freed by trypsinisation, encapsulated in alginate and cultured for a further 2 weeks. Rates of oxygen consumption and of lactate production were measured after cell isolation, during monolayer culture and then 2 weeks after return to 3D-culture and compared to those of chondrocytes cultured in alginate throughout.
Results: Expansion in monolayer increased energy consumption rates significantly. Oxygen consumption and lactate production rates for cells in monolayer were c.100 nmol/million cells/hr and 400 nmol/million cells/hr respectively, compared to 10 nmol/million cells/hr and 260 nmol/million cells/hr respectively for cells cultured in alginate beads throughout. The rates of metabolism had not recovered two weeks after return to 3-D culture.
Conclusions: The results showed that chondrocytes cultured in monolayer have a higher demand for nutrients than those in situ. Rates do not recover even when the chondrocytes are returned to a 3-D culture system. This has important implication for therapies using cells expanded in monolayer culture. If the nutrient supply is not sufficient, the cell viability and activity could be compromised.

4.4

Chondrocyte aggregation in suspension culture is mediated by Integrins

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Purpose: Chondrocytes aggregate in suspension culture and maintain their differentiated state. Since cell-cell and cell-matrix interactions influence phenotype, this study was performed to shed light on molecular interactions mediating chondrocyte aggregation.
Methods and Materials: Expression of selected cell adhesion molecules (CAM) including NCAM, N-Cadherin, and Integrins were studied by quantitative Real-Time PCR and Western-Blot, as well as several Integrin ligands in freshly isolated chondrocytes cultured serum-free in suspension. In parallel, potential inhibition of aggregation by blocking antibodies and peptides, chelating agents and enzymes directed against the ECM, were investigated. Cell aggregates were immunostained to identify potential receptor-ligand pairs implicated in aggregation.
Results: High expression of integrins containing the β1 subunit, combined with low levels of N-cadherin and NCAM suggested a primary role for β1 integrins in the aggregation of freshly isolated chondrocytes and therefore an ECM ligand. The presence of type II collagen in the pericellular regions of aggregates, along with the inhibition of aggregation by collagenase suggests type II collagen is implicated in integrin mediated aggregation. However aggregation was also found to be RGD dependant while direct interactions between fibrillar collagens and integrins (α1β1, α2β1, α10β1 or α11β1 integrins) are not RGD-dependent, suggesting a role of an associated protein such as COMP (cartilage oligomeric matrix protein).
Conclusions: Chondrocyte aggregation is mediated by an integrin containing the β1 sub-unit as cell receptor and by a collagen or a collagen-associated protein. Additional candidate ligands are being studied along with specific α sub-units.

4.5

Comparison of 10% autologous human serum compared to 10% fetal bovine serum on chondrocytes growth and formation of 3 dimensional cartilage-like structure

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Purpose: The use of 10% Fetal bovine serum (10% FBS9 in cell culture have been established to be the standard. The risk of certain diseases prompt us to evaluate the use of autologous human serum (10% AHS9 for chondrocyte growth and formation of a 3 dimensional structure using fibrin.
Methods and Materials: Cartilage specimens obtained from donors are processed using a standard protocol. Each sample is divided into 2 groups, using 10% FBS and 10% AHS. Monolayer culture were determine for growth rate, population doubling time and total cell doubling at each passage. For 3 dimensional construct, fibrin scaffold is used and was then implanted subcutaneously in a nude mice for maturation. Both monolayer culture and matured construct were histologically and genetically evaluated for comparison.
Results: There was significant difference in growth rate, population doubling time and total cell doubling. Histological assessment using Sfranin O staining showed presence of proteoglycans and gross histoarchitecture of the matured construct was glistening white and firm comparable to native articular cartilage. Phenotypic expression of Type 2 collagen was also seen in the 3 dimensional construct using one step RT-PCR technique.
Conclusions: The study showed autologous human serum is viable for chondrocyte growthand construct formation and may used for human transplantation.